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Ecological methods of observing and quantifying soil fungi

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Summary

Ecological methods of observing, isolating and quantifying fungi of soil, including those of plant litter and rotting wood, are reviewed. Progress in the last two decades is also briefly considered, and a combination of approaches recommended.

Introduction

Countless methods related to the study of the ecology of soil fungi have been described in the world's literature, since the arousal of interest in the subject about 100 years ago. How then should the choice of method be made when setting out to solve some new mycological problem? What advice can be given to the Ph.D. student about to investigate the fungi of a new habitat? This brief review, which considers soil in its broadest sense, i.e., including leaf litter and rotting wood, can only set the scene for thought on further developments. It does not include description of any magical new methods, nor does it cover the measurement of fungal activity—the essential end-point of many ecological investigations. For a discussion of the latter and for more details of the present subject, the reader is referred to Frankland et al. (in press). Unfortunately, the references are biased towards western literature as a result of language barriers.

Soil being a notoriously difficult medium in which to work, techniques for investigating a soil mycoflora are still far from ideal, and the choice depends heavily on compromise and a combination of approaches. S. D. Garrett, professor at Cambridge University for many years, said of soil fungi in 1952 that we see what we cannot identify, and identify what we cannot see. After nearly forty years, this statement still rings true. We see mycelium in soil that never appears in a Petri dish and we identify colonies in culture without knowing their origin. Their form and coloration may be beautiful and distinctive, but they are usually only artefacts of the laboratory. At first sight, many hyphae are featureless, and the active, dormant and dead states often indistinguishable. These are well-known facts but worth reiterating.

Progress over Two Decades, 1969–1989

Among a wealth of publications on new and standard methods, a few collective texts have held the stage for some years, e.g., Parkinson et al. (1971), Booth (1971) and Johnson and Curl (1972). The first was a genuine attempt to select the best methods for the International Biological Programme (IBP; 1967–1972), and to encourage global standardization, although there was a paucity of tropical information to draw on. This handbook was adopted rather late in the programme, but it stimulated international links, so that some comparison of IBP data has been possible (e.g., Kjøller and Struwe, 1982). The individual choice of technique has of course always depended on the aim, and the procedure has nearly always needed tailoring to the circumstances.

Although many classic techniques continue to be used, advances have been made, particularly during the last decade and particularly in the approach to a problem. The mycologist's concept of 'soil', for example, has widened considerably, so that both saws and trowels are needed. In the 1950s, incredible as it may seem now, plant litter was often scraped away before sampling a soil profile for fungi. The IBP helped to alter this attitude by emphasizing the importance of litter in nutrient cycling, but decomposition of non-commercial wood and Basidiomycotina as an ecological group were still somewhat neglected. The upsurge in research on saprotrophic fungi of plant litter and wood is evident from recent publications (e.g., Frankland et al., 1982; Rayner and Boddy, 1988a). The dramatic, world-wide increase in mycorrhizal research, after a dormant period (see Harley and Smith, 1983), has also brought improved mycological techniques with it. Mycorrhizal and other root fungi can no longer be separated rigidly from the so-called 'soil fungi' when it is known that their hyphae often extend considerable distances into the soil. In many soils, the most abundant mycelium could be that of *Glomus*.

Particularly within the laboratory, there have been important advances in technology, including, for example, those in microscopy and in analytical procedures such as high-performance liquid chromatography (HPLC). Sophisticated computers now enable both laboratory and field systems to be modelled, although the basic fungal data for the latter are usually far from adequate. The mycological content was, not unexpectedly, sparse in a recent symposium volume that was intended to highlight new field methods for use in nutrient cycling studies (Harrison et al., 1990).

In contrast to modern technology, quite simple observational methods have advanced the understanding of mycelium considerably. The ability to recognize individual mycelia has been the fundamental springboard of many new lines of research (Cooke and Rayner, 1984; Jennings and Rayner, 1984; Rayner and Boddy, 1988a). Mycologists now look for directional, flexible and co-ordinated fungal growth, or recognition responses between mycelia, rather than just featureless, ever-expanding mats of 'cotton-wool'.

An advance that has had a profound influence on the reputation and reliability of soil mycology as a science is the wider acceptance of the need to use statistical techniques in many different types of investigation. Some of the past 'giants' of mycology by their acute observations left a rich fund of information, but the more ecological and experimental aspects were underdeveloped through lack of a statistical approach. Computers have assisted the advance, allowing even non-numerate mycologists to tackle their problems statistically, although computer 'packages' can never replace entirely a good biologically-minded statistician. Checklists by Jeffers (1978, 1979, 1980), on the more significant questions to be taken into account when applying statistical methods, are excellent reminders of present-day requirements of well-designed experiments.

Direct Observation

Omitting measurement of fungal activity, methods of studying the ecology of soil fungi can be divided into three broad categories: direct observation, isolation techniques and quantification. Direct observation of the fungus in the field is a prerequisite of all ecological investigations, answering fundamental questions before the experimental stage. Where is the fungus? Is there a pattern? What is its resource? Which other organisms are associated with it? We should not be apologetic about the simplicity of the approach. In the rush for sophisticated equipment, microhabitats are easily lost sight of or destroyed altogether.

Macroscopic structures seen by the naked eye, such as fruit bodies, rhizomorphs and cords, can be valuable clues to the location of mycelium in soil and wood. For instance, cords like those of *Tricholomopsis platyphylla* (Pers. : Fr.) Singer have been uncovered and successfully traced through forest litter to determine the territory occupied and exploited (Thompson, 1984). Other features seen without recourse to a microscope include pigmented zone lines in litter and wood, evidence of interactions between individual mycelia and the development of fungal communities (Cooke and Rayner, 1984; Rayner and Boddy, 1988b).

The more ephemeral structures, particularly fleshy fruit bodies lasting only a few days, may indicate the nature of the nutrient resource but not the extent of the mycelium, or population and community structure, without repeated recording and mapping. Single rings of mycorrhizal fruit bodies around a host tree are well-known phenomena seen at a glance. More revealing are the complex patterns of fruiting found by mapping such species over a long period. From the use of simple (even if laborious) recording procedures, it is now known that spatial and temporal successions of mycorrhizal fungi are a common feature of many trees, the rings and spurs of fruit bodies reflecting the distribution of roots of various ages (e.g., Mason et al., 1982; Last et al., 1984, 1987).

The distribution of the mycelia of certain saprotrophic agarics has also been discovered by mapping fruit bodies, and then tracing them to their origin or investigating their genetic make-up (Newell, 1984; Frankland, 1984). Mapping alone can be misleading and the broader, regional surveys of fruiting agarics are particularly difficult to interpret. Arnolds (1988) has recorded interesting long-term changes in the macromycete flora of the Netherlands. They may indicate pollution effects, but the evidence is indirect and not necessarily related to the abundance of mycelium (see also p. 95).

If the fungi are only in the vegetative state, they can often be stimulated to grow and spore, without removal from the resource, by incubating the samples in a moist atmosphere, thus avoiding selective culture media. Bulky pieces of rotting wood can be wrapped in polythene, allowing mycelia to grow out, often luxuriously, so that they can be removed for identification and related to different zones and columns of decay (Rayner and Boddy, 1988a, b). Leaf litter and other organic material can be placed in a damp chamber, such as a Petri dish with a base lining of filter paper and a rim of highly absorbent, compressed paper kept slightly damp (Keyworth, 1951). Within about two days the arrangement of sporangiophores and conidiophores can be seen and spores are soon easily removed on an agar-tipped needle for identification. This very simple, early method can be extremely rewarding provided only 'resident' species are recorded. The chambers can be retained for some weeks to allow basidiomycetes to fruit, but contaminant microfungi may have appeared by then.

For observing in any detail the more microscopic fungi and mycelium hidden in its resource, more disturbance and use of microscope preparations are inevitable. Metallurgical lenses and phase-contrast microscopy can be useful aids, but the mycologist usually resorts to a battery of noxious chemicals for clearing and staining, and subjects the substrata to violent mechanical disruption in attempts to unmask the fungi. Pounding in mortars and severing in homogenizers are common practices, destroying all vestiges of the microhabitats! Kubiena (1938) avoided gross disturbance in his classic and elegant studies of fungi in soil by attaching a specially adapted microscope to the side of soil pits. This early work revealed important but, from our viewpoint, limited information. Today, sophisticated and expensive rhizotrons are an extension of this approach (Huck and Taylor, 1982).

A great variety of methods for observing fungi microscopically, within soil or plant tissue but in the laboratory, followed Kubiena's pioneering researches. Many of these involve preparation of impressions, peels and sections of the materials. In general, their potential is limited in proportion to the labour. One of the best techniques for preparing a soil section to obtain a 3-dimensional picture of hyphae in their microhabitats is that of Anderson (1978), since the soil is frozen in the field before being embedded in gelatine and sliced with a knife. It is, however, unsatisfactory for certain mineral soils that lack sufficient air spaces for penetration by the gelatine.

Other in situ methods include isotopic labelling and autoradiography, but the latter can give disappointing results with field material because of interference from bacteria commonly associated with hyphae (Waid et al., 1973). Isotopic labelling is usually of more value in the measurement of metabolic processes. At present, immunological techniques probably hold out most promise for locating and 'recognizing' specific mycelium in field material (Bohlool and Schmidt, 1980). Eventually, polyclonal antisera and monoclonal antibody banks will surely be available to the ecologist. As yet, however, use of these methods is justified only in well-defined situations, because of the time-consuming preliminaries and standardisations required, and the complications of cross-reactions. A simple, brief introduction to some of the principles involved has been published by Mitchell (1985). Genetic probes are also a challenge for the future.

For details and recommendations regarding staining procedures when using light microscopy on both field and laboratory material see Gurr (1965), Clark (1981) and Frankland et al. (in press).

Achieving ever more resolution and depth of focus than the light microscope, electron microscopes, both transmission (TEM) and scanning (SEM), continue, with improvements, to expose more detail. Spectacular illustrations are familiar, but they have, arguably, aided the taxonomist, physiologist and geneticist more than the ecologist, partly because of the destructive preparation procedures and the necessarily small size of the samples. Electron microscopy has, however, been valuable in elucidating patterns of colonization and enzyme production in decayed wood and on roots (Rayner and Boddy, 1988a; Greaves and Campbell, in press). It has also explained the remarkable capture mechanisms of nematode-trapping fungi (Nordbring-Hertz, 1984). SEM has also been used successfully, in combination with electron microprobe analysis, to determine the chemical composition of fungi still intact on the resource.

Isolation Techniques

Direct methods of observation, despite their special value, give only a partial view of a soil mycoflora. Even at the descriptive stage of an investigation, some means of isolating the fungi, such as a trap, bait or culture medium in a Petri dish, is usually needed. Immediately, problems of selection and bias arise, leading to difficulties in interpreting the significance of the 'catch'.

Several types of fungal trap have been buried in soil, from simple glass fibres to containers of nutrient agar with capillary orifices (see Booth, 1971). The agar in the latter is separated from the soil by an air gap, said to be crossed by only active hyphae. However, lingering doubts remain as to whether these traps stimulate germination of spores and isolate only the faster-growing species, although they sometimes show up interesting seasonal differences in the comparative growth rates of common fungi such as *Mucor* and *Trichoderma*. Buried baits, e.g., cellulose film, chitin strips and hair, are, however, an accepted means of isolating nutritionally distinct groups of fungi from soil. A variety of culture media can also serve as baits. Tubes of a polysulphide medium, for example, have been used to select fungi capable of utilizing reduced sulphur compounds

(Wainwright and Killham, 1980). Again, these baits do not necessarily distinguish between active and inactive members of a mycoflora.

If macroscopic structures, such as fruit bodies, rhizomorphs, cords or sheet aggregations of mycelium are formed in the field, inocula can be obtained quite readily by picking the material from its substratum. It is then washed or surface sterilized before plating portions on a culture medium, with or without antibiotics. Chemical analysis of such samples of field mycelium is also possible. Useful hints on the handling of agaric tissue have been given by Watling (1981).

Few mycologists, other than Warcup (1957, 1959), have been successful in isolating soil fungi by picking out individual hyphae from soil. He must have had the mycological equivalent of 'green fingers' (the English term for a good gardener). Even he had a relatively low success rate as regards the proportion of viable hyphae among the total number plated. The soils may have contained many dead hyphae, otherwise the results suggest that hyphae are excessively damaged during extraction. Some relatively uncommon species, including basidiomycetes, have been obtained by this method, but many hyphae are too closely associated with organic material to be retrievable. Söderström and Erland (1986) attempted to improve the technique by selecting only those hyphae that were vitally stained by fluorescein diacetate, but the results compared unfavourably with those of less laborious methods.

If hyphae cannot be isolated by dissecting them out of soil or organic materials, they must be induced to grow out as visible colonies onto a culture medium. The soil dilution technique, described in many standard texts, has its uses in comparative studies, but, as has been said repeatedly, it favours heavy sporers. One of the best alternatives for obtaining a general (but not complete) overview of a soil community is the soil plate technique (Warcup, 1950, 1951). It is easy and quick to disperse small crumbs of soil over the base of a dish and cover with a broad-spectrum agar medium. Alternatively, large organic components can be cut into fragments (ca. 1–2 mm^3) and pressed from above into the solidified medium. The procedure avoids the loss of inoculum that occurs by dilution; bacteria are restricted by the depth of agar, and active hyphae grow out in advance of germinating spores.

More ecological information can be obtained by washing the soil and collecting the different inorganic and organic particles on a series of graded sieves before plating. The microhabitat of a species is then indicated by its distribution between different types of particle. Several washing systems have been described, but the principles remain the same (e.g., Parkinson and Williams, 1961; Bissett and Widden, 1972). Bååth (1988) advocated the collection of relatively small particles to restrict the number of species developing from a single particle.

The isolation methods so far mentioned provide lists of species occurring in a fungal community. The population structure of individual species can be gained by a more genetical approach. From studies of genetically variable populations of Ascomycotina and Basidiomycotina, it has been shown that fungal populations in, for example, a plot of woodland litter or a tree branch can be mapped by culturing isolates in pairs, and recording the interactions between them (see Cooke and Rayner, 1984; Rayner and Boddy, 1988a, for references). As in the field, different genotypes are delimited by somatic/vegetative incompatibility reactions, evident as pigmented zones and as mycelial barrages and troughs between colonies, whereas somatically compatible strains usually intermingle without apparent antagonism.

Quantification

Ecologists often need to quantify fungi, but meaningful absolute numbers are not attainable. As A. D. M. Rayner has emphasized (personal communication), naturally occurring fungal entitites cannot be treated as countable units. Mycelia are dynamic systems and counts are clues to life spans and colonization strategies but no more. Laboratory dilution-plate counts, as already indicated, are unrelated to amounts of mycelium. Counts of fruit bodies may be useful, however, in relation to dimensions such as weight and to various environmental factors, but they should be extended over long periods, because fruiting can be so variable.

Estimates of frequency of occurrence, derived from such data as numbers of isolation plates or resource units, are often needed for simple comparative purposes, and sometimes can be expressed on an area or volume basis. Indices of 'species diversity' can also be obtained from species richness (numbers of species) and abundance (of individual species) by mathematical analysis, but they should be capable of distinguishing between closely similar environments, otherwise the exercise can be pointless (see Holder-Franklin, 1986; Magurran, 1988).

Measurements of biomass are a basic and more informative requirement of many ecological investigations at both the ecosystem and microcosm level. 'Biomass' should by definition refer to living matter only, but, as there is some confusion in the literature, it should be specified as living, dead or total (living+dead) when there could be doubt.

Complete separation of hyphae from soil to obtain the content of total fungal biomass has apparently never been achieved satisfactorily. Only about 20% of the total can be obtained by wet sieving and density gradient centrifuging (Bingle and Paul, 1986). More drastic procedures cause excessive fragmentation. The most direct estimates of total biomass are, therefore, those obtained from measurements of hyphal length in thin preparations of soil or litter with well-dispersed hyphae, but assumptions have to be made. Hyphal lengths are converted to volume and mass by assuming that hyphae are perfect cylinders, and conversion factors for diameter, moisture content and relative density of the hyphae have to be used. These factors are of crucial importance, and can affect the final values by several magnitudes. They should, therefore, be re-estimated for every new set of circumstances, and not be borrowed from the literature.

Even so, errors are likely to occur and must be minimized as far as possible. Diameter, moisture content and density are all known to vary widely with growth conditions and age of the mycelium (van Veen and Paul, 1979; Newell and Statzell-Tallman, 1982). Hyphal diameter is particularly important, since it is squared in the calculation of biomass (Bååth and Söderström, 1979). Measurements should be made, therefore, on field-grown mycelium if possible. Density can be measured either by volume displacement in water (Lodge, 1987) or by isopycnic centrifugation in a silica gel gradient (Bakken and Olsen, 1983).

The amount of maceration needed to disperse the hyphae before length measurements are undertaken is also critical, and optima should be predetermined. Hutchinson and King (1989) and Hutchinson et al. (1989) have described how data can be successfully combined from successive extracts of short duration. Removal of detached mycelium after each extraction reduces the risk of its destruction during further maceration.

Within the limitations mentioned, the Jones and Mollison (1948) procedure for measuring hyphal lengths in soil and litter, with the modifications of Thomas et al. (1965), has stood the test of time remarkably well in comparison with more modern methods. Only simple equipment is needed for preparing weighed amounts of homogenized material in agar films of known thickness on a haemocytometer slide, but phase-contrast microscopy is now recommended (Frankland, 1975a).

The time factor is the principal drawback of the agar-film technique, but the labour can be cut considerably by optimizing the experimental design (Frankland et al., 1978). Often too few replicate samples are taken from the field, and an unnecessarily large number of subsamples are examined at the microscopy stage. The membrane filter technique (Hanssen et al., 1974; Sundman and Sivelä, 1978), in which suspensions are filtered, and the hyphae retained on the membranes, has the same objective but is more rapid. The length measurements have correlated well with those obtained from agar films, but lower absolute values (Bååth and Söderström, 1980) suggest less accuracy (i.e., in the closeness of the results to the true values). Results using fluorescent stains on the membranes (unsatisfactory on agar films), however, appear to reverse this finding (West, 1988). Further investigation of this aspect is needed. Meanwhile, the membrane filter technique is recommended for comparative purposes (see Elmholt and Kjøller, 1987; Bardgett, in press). Again, the factors for converting length to biomass are as important as the primary measurements.

A crude estimate of live biomass can be obtained by an extension of hyphal length measurements for total biomass. The proportion of the total hyphal lumen possessing cell contents is determined along the length of the hyphae, on a volume basis, using phase contrast (Frankland, 1975b). Vital staining with fluorescein diacetate can also be attempted in the membrane filter technique to obtain estimates of the proportion of living biomass (Söderström, 1977, 1979).

Fungal biomass has frequently been derived by analysing for components of hyphae that are virtually specific to fungi, but the method has severe limitations for general purposes (Matcham et al., 1984). The selected component should be quantitatively extractable and present in all parts of the mycelium, but the concentration invariably differs with the species, and with the age and physiological state of the mycelium. Chitin content has been measured in 'pure-culture' conditions such as wood colonized by a single species (Swift, 1973), but the method is unsatisfactory in the presence of animal chitin. Ergosterol is more specific and probably the best choice in localized situations (Seitz et al., 1979; Newell et al., 1988). Sensitive assay methods, such as HPLC, have certainly improved the efficiency (man-hours) of these analytical techniques but have not overcome all the problems of variability. With respect to components of even greater specificity, quantitative immunology has great possibilities, but general methods of using antisera and antibodies on field material have yet to be perfected.

Respiration provides an entirely different approach to the estimation of biomass. The substrate-induced respiration (SIR) method using selective inhibitors (Anderson and Domsch, 1973, 1978) is rapid and objective, but several assumptions have to be made again, and more elaborate equipment is needed than in microscopy (see Jenkinson and Ladd, 1981). Agreement between biomass estimates by SIR and other techniques has been variable and has depended to some extent on soil type (see Frankland et al., in press, for further discussion and information on respirometers).

Production of mycelium in the field (i.e., the live biomass produced over a period of time) usually causes more measurement problems than any other dimension, but the information is beloved by mathematical modellers. Net production can be assessed by consecutive sampling and summing of positive changes in biomass, but gross production can be obtained only if account is taken of biomass losses, caused by such factors as grazing and lysis. A measurement of gross production in the field has been obtained for a basidiomycete on *Quercus* leaf litter, by using a fluorescent brightener to follow biomass loss, but the system was a simple one with a single dominant on a single resource (Frankland, 1975c; Frankland et al., 1979). Finally, the accuracy of such estimates can be assessed only by looking at an ecosystem in its entirety, i.e., at inputs and outputs, and determining whether or not the annual supply of nutrients is sufficient to sustain annual production. Only then does one come face to face with the real world and its complexities.

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Literature cited

Anderson, J. M. 1978. The preparation of gelatine-embedded soil and litter sections and their application to some soil ecological studies. J. Biol. Educ. **12**: 82–88.

Anderson, J. P. E. and Domsch, K. H. 1973. Quantification of bacterial and fungal contributions to soil respiration. Arch. Mikrobiol. **93**: 113–127.

Anderson, J. P. E. and Domsch, K. H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. **10**: 215–221.

Arnolds, E. 1988. The changing macromycete flora in the Netherlands. Trans. Br. Mycol. Soc. **90**: 391–406.

Bååth, E. 1988. A critical examination of the soil washing technique with special reference to the effect of the size of the soil particles. Can. Bot. **66**: 1566–1569.

Bååth, E. and Söderström, B. 1979. The significance of hyphal diameter in calculation of fungal biovolume. Oikos **33**: 11–14.

Bååth, E. and Söderström, B. 1980. Comparisons of the agar-film and membrane-filter methods for the estimation of hyphal lengths in soil, with particular reference to the effect of magnification. Soil Biol. Biochem. **12**: 385–387.

Bakken, L. R. and Olsen, R. A. 1983. Buoyant densities and dry-matter contents of microorganisms: conversion of a measured biovolume into biomass. Appl. Environ. Microbiol. **45**: 1188–1195.

Bardgett, R. D. The use of the membrane filter technique for comparative measurements of hyphal lengths in different grassland sites. In: "Modern techniques in soil ecology," (ed. by Crossley, D. A., Jr.). Elsevier, Amsterdam, and Agric. Ecosyst. & Environ. (in press).

Bingle, W. H. and Paul, E. A. 1986. A method for separating fungal hyphae from soil. Can. Microbiol. **32**: 62–66.

Bissett, J. and Widden, P. 1972. An automatic multichamber soil-washing apparatus for removing fungal spores from soil. Can. Microbiol. **18**: 1399–1404.

Bohlool, B. B. and Schmidt, E. L. 1980. The immunofluorescence approach in microbial ecology. Adv. Microbiol. Ecol. **4**: 203–236.

Booth, C. (ed.) 1971. "Methods in microbiology," **4**. Academic Press, London.

Clark, G. (ed.), 1981. "Staining procedures," 4th ed. Williams and Wilkins, Baltimore.

Cooke, R. C. and Rayner, A. D. M. 1984. "Ecology of saprotrophic fungi," Longman, London.

Elmholt, S. and Kjøller, A. 1987. Measurement of the length of fungal hyphae by the membrane filter technique as a method for comparing fungal occurrence in cultivated field soils. Soil Biol. Biochem. **19**: 679–682.

Frankland, J. C. 1975a. Importance of phase-contrast microscopy for estimation of total fungal biomass by the agar-film technique. Soil Biol. Biochem. **6**: 409–410.

Frankland, J. C. 1975b. Estimation of live fungal biomass. Soil Biol. Biochem. **7**: 339–340.

Frankland, J. C. 1975c. Fungal decomposition of leaf litter in a deciduous woodland. In: "Biodégradation et humification," (ed. by Kilbertus, G. et al.), pp. 33–40. Pierron, Sarreguemines.

Frankland, J. C. 1984. Autecology and the mycelium of a woodland litter decomposer. In: "The ecology and physiology of the fungal mycelium," (ed. by Jennings, D. H. and Rayner, A. D. M.), pp. 241–260. Cambridge University Press, Cambridge.

Frankland, J. C., Bailey, A. D. and Costeloe, P. L. 1979. Ecology of a woodland toadstool, *Mycena* *galopus*. Ann. Rep. 1978, pp. 26–28. Institute Terrestrial Ecology, Grange-over-Sands, UK.

Frankland, J. C., Dighton, J. and Boddy, L. Methods of studying the ecology of fungi in soil and forest litter. In: "Methods in microbiology," (ed. by Norris, J. R.), **22**. Academic Press, London. (in press).

Frankland, J. C., Hedger, J. N. and Swift, M. J. (eds.) 1982. "Decomposer basidiomycetes: their biology and ecology," Cambridge University Press, Cambridge.

Frankland, J. C., Lindley, D. K. and Swift, M. J. 1978. A comparison of two methods for the estimation of mycelial biomass in leaf litter. Soil Biol. Biochem. **10**: 323–333.

Garrett, S. D. 1952. The soil fungi as microcosm for ecologists. Sc. Prog. **40**: 436–450.

Greaves, M. P. and Campbell, R. The microbial ecology of the rhizosphere. In: "Methods in microbiology," (ed. by Norris, J. R.) **22**. Academic Press, London. (in press).

Gurr, E. 1965. "The rational use of dyes in biology," Leonard Hill, London.

Hanssen, J. F., Thingstad, T. F. and Goksøyr, J. 1974. Evaluation of hyphal lengths and fungal biomass in soil by a membrane filter technique. Oikos **25**: 102–107.

Harley, J. L. and Smith, S. E. 1983. "Mycorrhizal symbiosis," Academic Press, London.

Harrison, A. F., Ineson, P. and Heal, O. W. (eds), 1990. "Nutrient cycling in terrestrial ecosystems: field methods, application and interpretation," Elsevier, London.

Holder-Franklin, M. A. 1986. Ecological relationships in water and soil as revealed by diversity measurements. In: "Microbial autecology. A method for environmental studies," (ed. by Tate III, R. L.), pp. 93–132. John Wiley, New York.

Huck, M. G. and Taylor, H. M. 1982. The rhizotron as a tool for root research. Adv. Agron. **35**: 1–35.

Hutchinson, K. J. and King, K. L. 1989. Volume and activity of micro-organisms in litter from native and sown temperate pasture species. Aust. Ecol. **14**: 157–167.

Hutchinson, K. J., King, K. L., Nicol, G. R. and Wilkinson, D. R. 1990. Methods for evaluating the role of residues in the nutrient cycling economy of pastures. In: "Nutrient cycling in terrestrial ecosystems: field methods, application and interpretation," (ed. by Harrison, A. F., Ineson, P. and Heal, O. W.), pp. 291–314. Elsevier, London.

Jeffers, J. N. R. 1978. "Design of experiments," Statistical checklist 1. Institute of Terrestrial Ecology, Grange-over-Sands, UK.

Jeffers, J. N. R. 1979. "Sampling," Statistical checklist 2. Institute of Terrestrial Ecology, Grange-over-Sands, UK.

Jeffers, J. N. R. 1980. "Modelling," Statistical checklist 3. Institute of Terrestrial Ecology, Grange-over-Sands, UK.

Jenkinson, D. S. and Ladd, J. N. 1981. Microbial biomass in soil: measurement and turnover. In: "Soil biochemistry," (ed. by Paul, E. A. and Ladd, J. N.) **5**: 415–471. Marcel Dekker, New York.

Jennings, D. H. and Rayner, A. D. M. (eds) 1984. "The ecology and physiology of the fungal mycelium," Cambridge University Press, Cambridge.

Johnson, L. F. and Curl, E. A. 1972. "Methods for research on the ecology of soil-borne plant pathogens," Burgess Publishing Co., Minneapolis.

Jones, P. C. T. and Mollison, J. E. 1948. A technique for the quantitative estimation of soil microorganisms. J. Gen. Microbiol. **2**: 54–69.

Keyworth, W. G. 1951. A Petri dish moist chamber. Trans. Br. Mycol. Soc. **34**: 291–292.

Kjøller, A. and Struwe, S. 1982. Microfungi in ecosystems: fungal occurrence and activity in litter and soil. Oikos **39**: 389–422.

Kubiena, W. L. 1938. "Micropedology," Collegiate Press, Inc., Ames, Iowa.

Last, F. T., Mason, P. A., Ingleby, K., Fleming, L. V. 1984. Succession of fruit bodies of sheathing mycorrhizal fungi associated with *Betula pendula*. For. Ecol. Manage. **9**: 229–234.

Last, F. T., Mason, P. A., Ingleby, K. and Fleming, L. V. 1987. Successions of sheathing mycorrhizal fungi. Trends in Ecology and Evolution **2**: 157–161.

Lodge, D. J. 1987. Nutrient concentrations, percentage moisture and density of field-collected fungal mycelia. Soil Biol. Biochem. **19**: 727–733.

Magurran, A. E. 1988. "Ecological diversity and its measurement," Croom Helm, London.

Mason, P. A., Last, F. T., Pelham, J. and Ingleby, K. 1982. Ecology of some fungi associated with an ageing stand of birches (*Betula pendula* and *Betula pubescens*). For. Ecol. Manage. **4**: 19–37.

Matcham, S. E., Jordon, B. R. and Wood, D. A. 1984. Methods for assessment of fungal growth on solid substrates. In: "Microbiological methods for environmental biotechnology," (ed. by Grainger, J. M. and Lynch, J. M.), pp. 5–18. Academic Press, London.

Mitchell, L. A. 1985. Monoclonal antibodies and immunochemical techniques: applications in forestry research. Information Report, Pacific Forest Research Centre, No. BC-X-258. Canadian Forestry Service, Victoria, B.C.

Newell, K. 1984. Interaction between two decomposer basidiomycetes and a collembolan under Sitka spruce: distribution, abundance and selective grazing. Soil Biol. Biochem. **16**: 227–233.

Newell, S. Y. and Statzell-Tallman, A. 1982. Factors for conversion of fungal biovolume values to biomass, carbon and nitrogen: variation with mycelial ages, growth conditions, and strains of fungi from a salt marsh. Oikos **39**: 261–268.

Newell, S. Y., Arsuffi, T. L. and Fallon, R. D. 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. Appl. Environ. Microbiol. **54**: 1876–1879.

Nordbring-Hertz, B. 1984. Mycelial development and lectin-carbohydrate interactions in nematode-trapping fungi. In: "The ecology and physiology of the fungal mycelium," (ed. by Jennings, D. H. and Rayner, A. D. M.), pp. 419–432. Cambridge University Press, Cambridge.

Parkinson, D. and Williams, S. T. 1961. A method for isolating fungi from soil microhabitats. Plant Soil **13**: 347–355.

Parkinson, D., Gray, T. R. G. and Williams, S. T. 1971. "Methods for studying the ecology of soil micro-organisms," International Biological Programme Handbook No. 19. Blackwell Scientific Publications, Oxford.

Rayner, A. D. M. and Boddy, L. 1988a. "Fungal decomposition of wood: its biology and ecology," John Wiley, Chichester.

Rayner, A. D. M. and Boddy, L. 1988b. Fungal communities in the decay of wood. Adv. Microb. Ecol. **10**: 115–166.

Seitz, L. M., Sauer, D. B., Burroughs, R., Mohr, H. E. and Hubbard, J. D. 1979. Ergosterol as a measure of fungal growth. Phytopathology **69**: 1202–1203.

Söderström, B. E. 1977. Vital staining of fungi in pure culture and in soil with fluorescein diacetate. Soil Biol. Biochem. **9**: 59–63.

Söderström, B. E. 1979. Some problems in assessing the fluorescein diacetate-active fungal biomass in the soil. Soil Biol. Biochem. **11**: 147–148.

Söderström, B. and Erland, S. 1986. Isolation of fluorescein diacetate stained hyphae from soil by micromanipulation. Trans. Br. Mycol. Soc. **86**: 465–468.

Sundman, V. and Sivelä, S. 1978. A comment on the membrane filter technique for estimation of length of fungal hyphae in soil. Soil Biol. Biochem. **10**: 399–401.

Swift, M. J. 1973. The estimation of mycelial biomass by determination of the hexosamine content of wood tissue decayed by fungi. Soil Biol. Biochem. **5**: 321–332.

Thomas, A., Nicholas, D. P. and Parkinson, D. 1965. Modifications of the agar film technique for assaying lengths of mycelium in soil. Nature (Lond.) **205**: 105.

Thompson, W. 1984. Distribution, development and functioning of mycelial cord systems of decomposer basidiomycetes of the deciduous woodland floor. In: "The ecology and physiology of the fungal mycelium," (ed. by Jennings, D. H. and Rayner, A. D. M.), pp. 185–214. Cambridge University Press, Cambridge.

van Veen, J. A. and Paul, E. A. 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. Appl. Environ. Microbiol. **37**: 686–692.

Waid, J. S., Preston, K. J. and Harris, P. J. 1973. Autoradiographic techniques to detect active microbial-cells in natural habitats. In: "Microbial methods in the study of microbial ecology," (ed. by Rosswall, T.). Ecol. Res. Comm. Bull. (Stockholm) **17**: 317–322.

Wainwright, M. and Killham, K. 1980. Sulphur oxidation by *Fusarium solani*. Soil Biol. Biochem. **12**: 555–558.

Warcup, J. H. 1950. The soil plate method for isolation of fungi from soil. Nature (Lond.) **166**: 117–118.

Warcup, J. H. 1951. The ecology of soil fungi. Trans. Br. Mycol. Soc. **34**: 376–399.

Warcup, J. H. 1957. Studies on the occurrence and activity of fungi in a wheat-field soil. Trans. Br. Mycol. Soc. **40**: 237–262.

Warcup, J. H. 1959. Studies on basidiomycetes in soil. Trans. Br. Mycol. Soc. **42**: 45–52.

Watling, R. 1981. "How to identify mushrooms to genus. V: cultural and developmental features," Mad River Press, Inc., Eureka, California.

West, A. W. 1988. Specimen preparation, stain type, and extraction and observation procedures as factors in the estimation of soil mycelial lengths and volumes by light microscopy. Biol. Fertil. Soils **7**: 88–94.

摘要

土壌菌類の観察及び定量のための生態学的手法

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植物の落葉及び腐朽木を含む土壌中の菌類を観察し，分離し，定量するための生態学的手法を総説した．同時に最近20年間の進歩を簡単に考察し，研究アプローチを示した．